Immunoreactive proteins of *Brachyspira hyodysenteriae* in pigs

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Introduction

Swine dysentery (SD), caused by the spirochete *Brachyspira hyodysenteriae*, is a major problem in swine industry worldwide. Few antimicrobial drugs are active against *B. hyodysenteriae* and acquired antimicrobial resistance is regularly reported. The introduction of asymptomatic carrier animals represents an important threat for negative herds. Diagnosis is based on clinical signs, culture and PCR. However, no reliable diagnostic tests are commercially available to verify the SD status of a herd. By use of two-dimensional gel electrophoresis and western blotting (2DWB) followed by mass spectrometry (MS) we aimed to identify immunoreactive proteins of *B. hyodysenteriae* that could be useful for the development of a serological diagnostic test.

Materials and Methods

A total protein extract of *B. hyodysenteriae* was separated by a 2DWB protocol. Immunoreactive proteins were visualized by incubation of the blot with sera from pigs that showed clinical signs of SD after experimental inoculation with *B. hyodysenteriae*. Spots of interest were excised from the protein gel and digested with trypsin prior to MS. In a modified 2DWB protocol, serum of an infected pig was first incubated with a pool of total protein extracts of *B. innocens*, *B. intermedia*, *B. pilosicoli*, *B. murdochii* and "*B. hampsonii*" to capture antibodies that also reacted with proteins from these species, thus selecting for antigens specific to *B. hyodysenteriae*.

Results

The 2DWB, with sera from experimentally infected pigs, followed by MS resulted in a comprehensive list of potentially immunoreactive proteins of *B. hyodysenteriae*. However, total protein extracts of other *Brachyspira* species showed important similarities to that of *B. hyodysenteriae*. Preabsorption of the primary serum with total protein extracts of these *Brachyspira* species resulted in a less extensive pattern on the blots compared to the regular 2DWB protocol offering a solution to this problem.

Conclusion

Asymptomatic carrier animals are an important risk factor for the spread of SD between herds. A serological test could detect these dormant sources of SD and facilitate diagnosis and prevention. A drawback in the search of proteins unique for *B. hyodysenteriae* is the similarity between *Brachyspira* species. Using our adapted 2DWB protocol followed by MS we were able to compensate for this problem and identify proteins of *B. hyodysenteriae* that are potential candidates for the development of diagnostics. Further research is needed to confirm the importance of these proteins of *B. hyodysenteriae* by recombinant expression followed by one-dimensional gel electrophoresis.